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The Molecular Structure of Polyadenylic Acid

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The structure of fibers of polyadenylic acid at acid pH has been studied by X-ray diffraction. A model is proposed consisting of two parallel intertwined helical chains, each having a screw of 3·8 Å and 45° and related to each other by a dyad axis parallel to the fiber axis. Coordinates, bond distances and angles and the calculated Fourier transform are given for this model.

Reasons are given why the quite different model of Morgan & Bear is thought to be wrong.

1. Introduction

The isolation of polynucleotide phosphorylase by Grunberg-Manago, Ortiz & Ochoa (1955) has made possible the synthesis of a variety of ribonucleotide polymers all having the same covalent backbone as natural RNA. Physical and chemical studies on these polymers have been widespread and have led to a better understanding of the general principles underlying nucleic acid structures. Among the first of these polymers to be investigated by the technique of X-ray diffraction was polyadenylic acid. In this paper we describe our studies of this material and give the precise coordinates for a molecular model which is in excellent agreement with all the experimental facts.

Preliminary accounts of this work have appeared previously (Watson, 1957; Rich, 1957*a*; Crick, 1957). Since that time additional information has been obtained from the physical chemical investigations of Beers & Steiner (1957), Fresco & Doty (1957) and Fresco (1959), and the results of these investigations will be incorporated into the present discussion.

2. Materials and Methods

The polyadenylic acid originally used in this investigation was kindly supplied by S. Ochoa. Later preparations were synthesized by methods already described. A large number of preparations was investigated and the results were very similar in all cases except that some specimens of low molecular weight could not be oriented.

Fibers were pulled from concentrated solutions of polyadenylic acid and X-ray diffraction photographs of these fibers were obtained with fiber micro-cameras. The humidity of

the helium atmosphere surrounding the fibers was controlled by bubbling the helium through appropriate saturated salt solutions before it entered the camera. Both the microfocus X-ray tube (Hilger, London) and the rotating anode tube which has been developed at the Cavendish Laboratory, Cambridge, were used as high intensity X-ray sources. The intensities of the diffraction maxima on the photographs were measured with a Joyce-Loebl recording microdensitometer.

The structural investigation was carried out with the aid of molecular models built from 1/8 in brass rod to a scale of 5 cm = 1 Å. Models were built of plausible helical structures and from these models a set of coordinates could be obtained for the repeat unit of the helix. In the early stages the diffraction pattern was obtained using an optical transform machine. Later, IBM computers were used to calculate from the coordinates the cylindrically averaged Fourier transform of the model. This calculation has been described previously (Davies & Rich, 1959). Comparison between observed and calculated diffraction patterns was then made, and from this a decision could be reached as to the acceptability of the model. The coordinates of the final model were then refined so that all distances and angles were stereochemically acceptable.

3. Results

Well oriented fibers of polyadenylic acid are negatively birefringent with values up to $\Delta n = -0{\cdot}10$. Some less well oriented fibers produced smaller values of the birefringence but always of negative sign. Some preparations produced well oriented samples while others yielded poor orientation. The degree of orientation was best correlated to the molecular length of the polymer, and the pH of the material. If the sample had been prepared near neutral pH, it was very difficult to get a well oriented sample; however, lowering the pH usually resulted in a sample with more orientation.

An X-ray diffraction photograph of polyadenylic acid is shown in Plate I. This is a typical fiber diffraction photograph characterized by a series of off-equatorial streaks rather than a number of discrete spots such as one would expect for a crystalline material. The diffraction photograph is dominated by a sharp, very intense meridional reflexion at 3·80 Å on the fourth layer line. The first layer line in the photograph has a spacing of 15·2 Å, and has a medium to weak reflexion near the meridian; further out there is a moderately strong reflexion at a Bragg spacing of 5·2 Å. The latter reflexion shows some arcing and is not confined entirely to the first layer line, but merges with a reflexion on the equator. It is of interest to note that a similar reflexion also occurs in the diffraction pattern of natural ribonucleic acid fibers (Rich & Watson, 1954). The second layer line has nothing while the third layer line has a reflexion which is moderately weak and is located just off the meridian. Sometimes this layer line can be broken up into two reflexions, one of which is closer to the meridian than the other. The fourth layer line has, in addition to the very intense meridional reflexion, a suggestion of much weaker components which are further away from the meridian and blurred. The fifth layer line has a reflexion of moderate intensity which is off meridional but arcs across it.

In addition to the above reflexions which can be seen with untilted fibers, there are two layer line reflexions which can only be seen by tipping the specimens. An example of a tipped diffraction pattern is shown in Plate II. As can be seen, there are two near-meridional reflexions which occur at a Bragg spacing of 1·90 Å and 1·68 Å. It should be noted that the 1·90 Å reflexion is most intense on the meridian while the outer 1·68 Å reflexion covers a somewhat wider arc.

Diffraction photographs were taken of polyadenylic acid at a variety of relative humidities in order to determine the influence of water on the structure. One of the

PLATE I. An X-ray diffraction photograph of polyadenylic acid. The fiber axis is vertical and the relative humidity is 78%. The weak reflexion on the meridian just inside the intense 3·8 Å arc is due to Kβ radiation.

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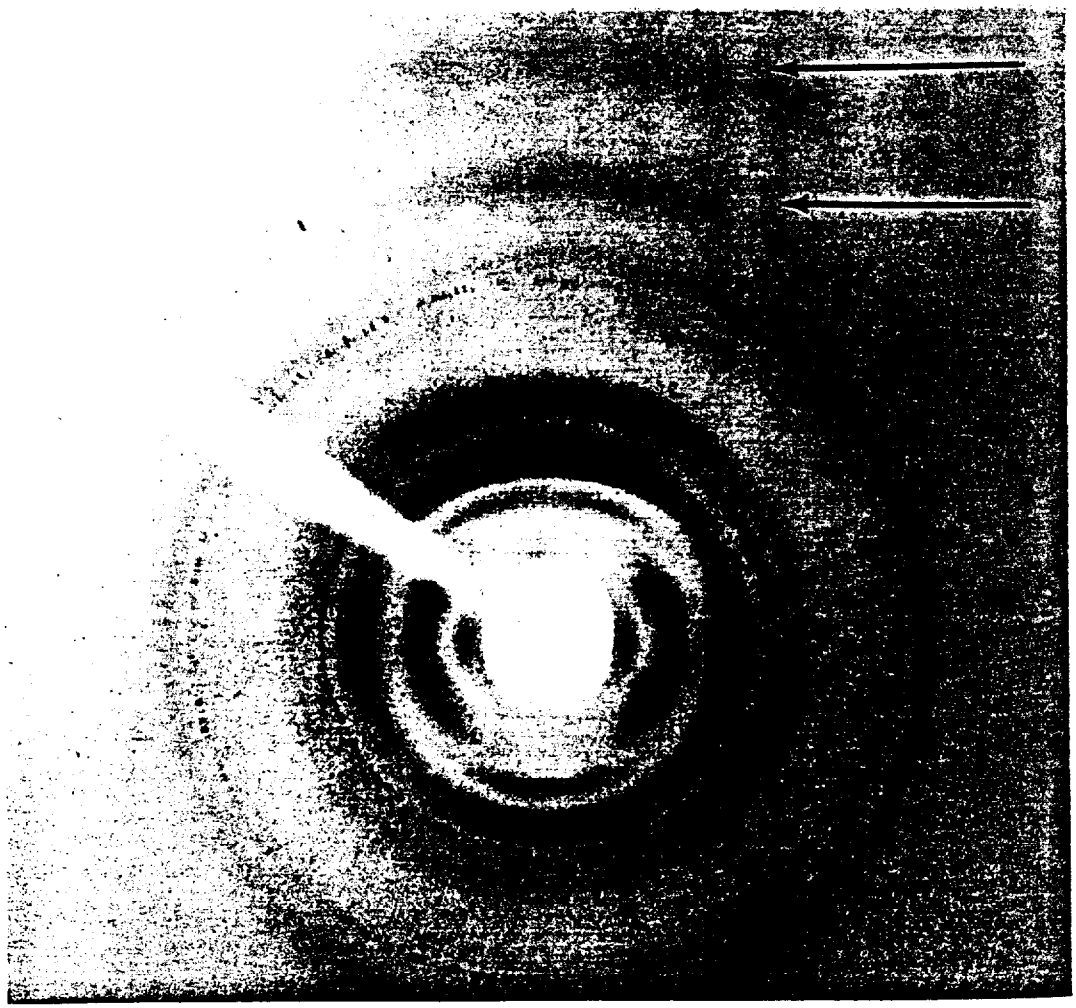

PLATE II. An X-ray diffraction photograph of polyadenylic acid obtained with the fiber axis vertical, but tipped about 20° into the beam. Two additional strong reflexions are seen as shown by the arrows.

most unusual features of polyadenylic acid is the fact that the diffraction pattern remains intact even if the photograph is taken with the specimen in an evacuated camera at zero relative humidity, so that almost all the labile water molecules are removed. This stability is in marked contrast to deoxyribonucleic acid and most of the other synthetic polyribonucleotides, in which the diffraction pattern becomes amorphous or blurred when the fiber is put in a vacuum. The variations in relative humidity have only a small effect on the equatorial diffraction pattern of polyadenylic acid. The first reflexion on the equator, 100, is very strong and occurs at a spacing between 16 and 18 Å. The 100 spacing is shown for polyadenylic acid as a function of relative humidity in Fig. 1, and it can be seen that there is only a small change of less

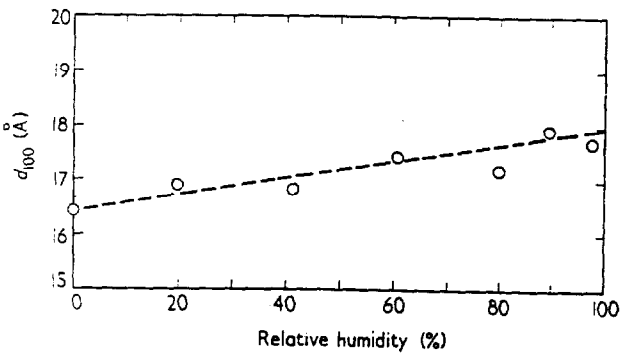

FIG. 1. The change in the 100 reflexion with changes in relative humidity. Zero relative humidity was produced by taking the diffraction photograph under vacuum.

than 2 Å in this spacing over the whole range of relative humidity. In the two-stranded molecule of polyadenylic acid *plus* polyuridylic acid, the 100 spacing increases by over 8 Å when the relative humidity is changed from near zero to 98% (Rich. 1957*b*).

The reflexions on the equator are not particularly well defined and a degree of uncertainty must be attached to their spacings. They were measured with the Joyce-Loebl recording microdensitometer from twelve films which were the product of 10 separate photographs at different relative humidities. In general, only two reflexions could be seen easily, having a spacing of about 17 Å and about 7·6 Å. On several films a reflexion of spacing near 8·6 Å could be seen, and on two films a fourth faint reflexion could be observed, but not measured.

The first two reflexions may be tentatively indexed as the 100 and 210 reflexions from a square net of spacing 17 Å. The third would then correspond to 200 and the fourth to 110. It seems to us most unlikely that these could be the result of diffraction from a hexagonal net, but in view of the paucity of the equatorial reflexions, and the rather diffuse nature of those which are observed, their assignment to a tetragonal cell is not entirely certain.

4. Interpretation of the Diffraction Pattern

The strong negative birefringence of the fibers of polyadenylic acid suggested that the purine bases are oriented more or less at right angles to the fiber axis. This interpretation was reinforced by the extremely strong meridional reflexion at 3·8 Å.

However, since the thickness of the purine bases is 3·4 Å, it is clear that they must be tilted a little in order to explain the observed 3·8 Å axial spacing.

The distribution of meridional and off-meridional reflexions clearly suggests that the molecule is helical in character. The simplest helix that is compatible with this distribution would be one in which there are four residues per turn with a pitch of 15·2 Å. Accordingly, we spent some time investigating configurations of a variety of single-stranded polyadenylic acid models, since we were at that time uncertain of the diameter of the molecule. We tried to find a natural explanation for the 3·8 Å reflexion, and in addition to normal stereochemistry, we looked for a reasonable system of hydrogen bonding to hold the structure together. After considerable effort in this direction, we concluded that it was impossible with a single-stranded molecule.

Shortly after this we obtained somewhat more reliable data on the equatorial reflexions of polyadenylic acid which indicated that there was a larger unit cell having an edge about 16 to 17 Å long. It was clear that a single strand of polyadenylic acid could not easily fill a volume of this sort and accordingly we then began to investigate multi-stranded helical models.

In general, there are two different classes of multi-stranded structures: those in which the purine residues are located in the center of the molecule, and those in which they are located on the outside of the molecule. Investigations of the configurations possible in the latter class led us to the conclusion that there were none that were plausible. This point will be discussed in greater detail when we consider the model advanced by Morgan & Bear (1958).

Investigation of multi-stranded models with the bases on the inside showed that the ribose-phosphate backbone is not long enough to cover the distance required for a translation of 3·8 Å and a rotation of 90° which was indicated by the strong meridional reflexion on the 4th layer line. However, two-stranded models can be built with a twofold rotation axis running down the helix axis. The effect of this symmetry axis is to reduce the unit rotation to 45°, and within this framework there are two structures in which the adenines of opposite chains are joined together in pairs by hydrogen bonds.

In the first of these structures the 6-amino group is hydrogen bonded to N_1 of the opposite purine ring. Models of this type were rejected by us because they require a large radial separation between phosphates of opposite chains. This would lead to strong reflexions near the meridian on the first layer line, contrary to what is observed. They were examined in more detail by Morgan & Bear (1958), and rejected for similar reasons.

The second of these structures has the adenines paired in the same way as in the crystal structure of adenine hydrochloride (Broomhead, 1948; Cochran, 1951), namely, with hydrogen bonds between the 6-amino nitrogen and N_7 of the opposite ring. A first primitive model of this sort had the bases stacked perpendicular to the helix axis with a separation of 3·8 Å, and no attempt was made to pull in the ribose phosphate backbones closer to the axis. The calculated diffraction pattern showed reasonable agreement with the observed pattern, although the intensities of the first layer line reflexions were reversed, the first being strong and the second weak.

However, at the same time a second model of this kind was constructed in which an additional hydrogen bond was made between the 6-amino nitrogen and a phosphate oxygen of the opposite chain. This additional hydrogen bond meant that the two strands were now held together by two purine-purine hydrogen bonds and two

purine-phosphate hydrogen bonds. Formation of this additional hydrogen bond results in pulling in the phosphates closer to the helix axis with consequent improvement of the agreement between the diffraction patterns. Furthermore, in order to make this bond, it is necessary to tip the bases so that they are no longer perpendicular to the helix axis, but inclined by 10° to 11° from the perpendicular. This tilting provides a natural explanation for the 3·8 Å spacing rather than the expected 3·4 Å spacing.

TABLE 1

Coordinates of polyadenylic acid

(The atoms listed are those illustrated in Fig. 5)

	x	y	z	r	ϕ
Adenine residue					
N_1	1·77	3·68	0·35	4·08	64·3°
C_2	3·14	3·73	0·46	4·88	49·9°
N_3	3·92	2·70	0·35	4·76	34·5°
C_4	3·24	1·54	0·11	3·59	25·5°
C_5	1·89	1·39	− 0·02	2·35	36·3°
C_6	1·08	2·52	0·10	2·74	66·7°
N_7	1·59	0·08	− 0·27	1·59	2·7°
C_8	2·80	− 0·50	− 0·27	2·84	− 10·1°
N_9	3·81	0·33	− 0·05	3·82	4·9°
N_{10}	− 0·21	2·55	0·01	2·56	94·7°
Ribose-phosphate chain					
C'_1	5·24	0·00	0·00	5·24	0·0°
C'_2	5·69	0·29	1·44	5·70	2·9°
C'_3	5·58	− 1·09	2·09	5·69	− 11·0°
C'_4	6·04	− 2·02	0·98	6·37	− 18·5°
C'_5	5·51	− 3·45	1·19	6·51	− 32·1°
O'_1	5·48	− 1·42	−0·21	5·66	− 14·5°
O'_2	7·03	0·78	1·48	7·08	6·3°
O'_3	6·46	− 1·22	3·26	6·58	− 10·7°
P	3·34	− 4·92	0·89	5·95	− 55·8°
O_5	4·07	− 3·53	1·00	5·39	− 40·9°
O_6	1·86	− 4·65	0·90	5·01	− 68·2°
O_7	3·89	− 5·91	1·89	7·07	− 56·6°
lower O'_3	3·71	− 5·45	− 0·54	6·58	− 55·7°

A right-handed coordinate system is used for both Cartesian and cylindrical polar coordinates.

Furthermore, tilting the bases alters the distribution of scattering intensity on the upper layer lines. If the bases were not tilted we would only expect higher orders of 3·8 Å, e.g. the 1·9 Å reflexion which is observed as moderately strong (Plate II). However, tilting also throws some diffracted intensity somewhat off the meridian so that we might expect additional reflexions to occur in that region. We have pointed out the occurrence of a second strong reflexion at 1·68 Å which, as we will see below, is predicted by this model.

By hydrogen-bonding the phosphate group to the adenine residue of the opposite chain, the molecule tended to become more rounded in cross section, and the helical

grooves, which are so marked in DNA, are considerably reduced in polyadenylic acid. This has the effect of reducing the predicted intensity of the second layer line near the meridian so that it is in closer agreement with the observed diffraction pattern.

5. Description of the Molecule

Once we were convinced that this model was along the right general lines for predicting the overall diffraction pattern, we carried out a series of successive adjustments whereby a careful set of atomic coordinates was obtained which showed that it is stereochemically feasible to have a model of this sort. Thus, we have a set of atomic

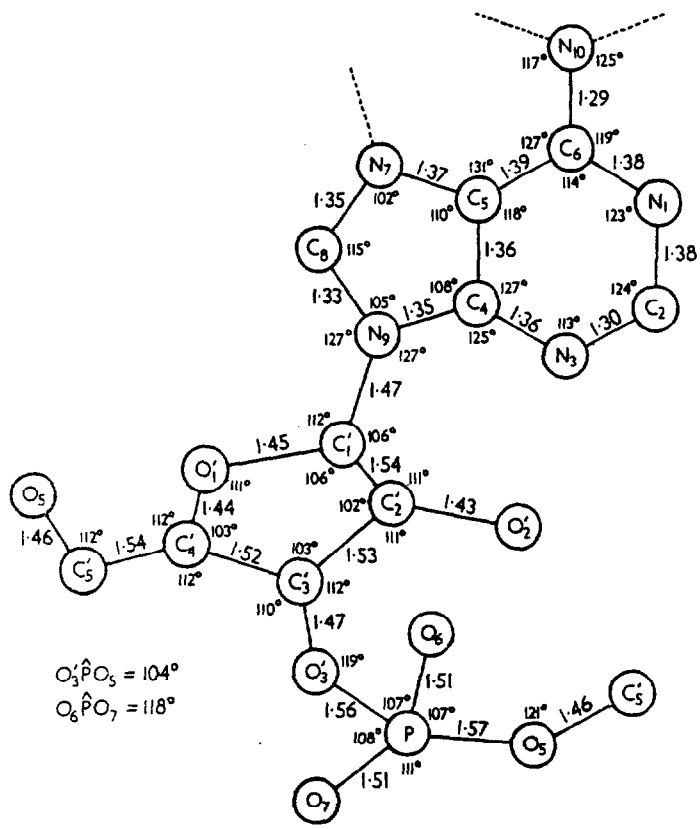

FIG. 2. A sketch to show the bond angles and distances of polyadenylic acid. Not to scale.

coordinates which yield an accepted range of values for bond angles, distances, and van der Waals contacts between the atoms. The coordinates for polyadenylic acid are listed in Table 1, the bond distances and angles are shown in Fig. 2 (not to scale), and the shorter van der Waals contacts are marked on the projection of part of the structure shown in Fig. 3.

The coordinates were finalized before the publication of the papers of Fuller (1959) and Spencer (1959). They could be revised slightly with advantage but all the distances and angles are acceptable, although the angle $\widehat{N_9C_1'C_2'}$ (106°) is strained a little owing to the formation of the hydrogen bond between N_{10} and O_6. The adenine has been based on Cochran's parameters for adenine hydrochloride (Cochran, 1951).

The hydrogen bond from N_{10} to N_7 is 6° away from the straight direction and that from N_{10} to O_6 is 14° away; that is, the angle $O_6\hat{N}_{10}H$ is 14°. This is on the large side, but acceptable.

It should be mentioned here that it is necessary in the determination of molecular structure from fiber patterns to show that a given molecular model is stereochemically

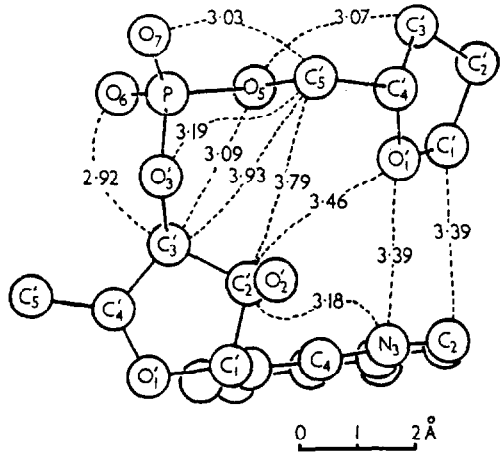

FIG. 3. Part of the structure projected in the direction of the x axis. The shorter van der Waals contacts are shown by dotted lines.

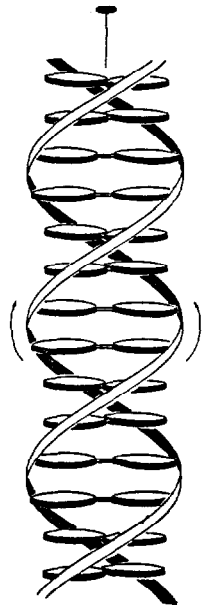

FIG. 4. Schematic view of polyadenylic acid as seen from the side. The flat ribbons represent the ribose-phosphate backbones which are parallel to each other. The disks represent the adenine rings which are hydrogen bonded together.

feasible, and to present reasonably exact atomic coordinates. This is not because it is necessary to compare in great detail the diffraction pattern of this refined model with what is observed experimentally, for very often the experimental data on an X-ray

fiber diffraction pattern are not of high quality. The importance of presenting exact coordinates rests on the fact that this is a means for ruling out certain structures. It may be very easy to obtain an approximate set of coordinates, but it may prove to be impossible to refine the model properly so that it conforms to known values of atomic parameters as well as have the symmetry demanded by the diffraction pattern.

The helical molecule of polyadenylic acid consists of two polynucleotide chains organized about a twofold rotation axis. Figure 4 is a schematic view of the molecule in which the flat ribbons represent the ribose-phosphate backbone chains and the disks

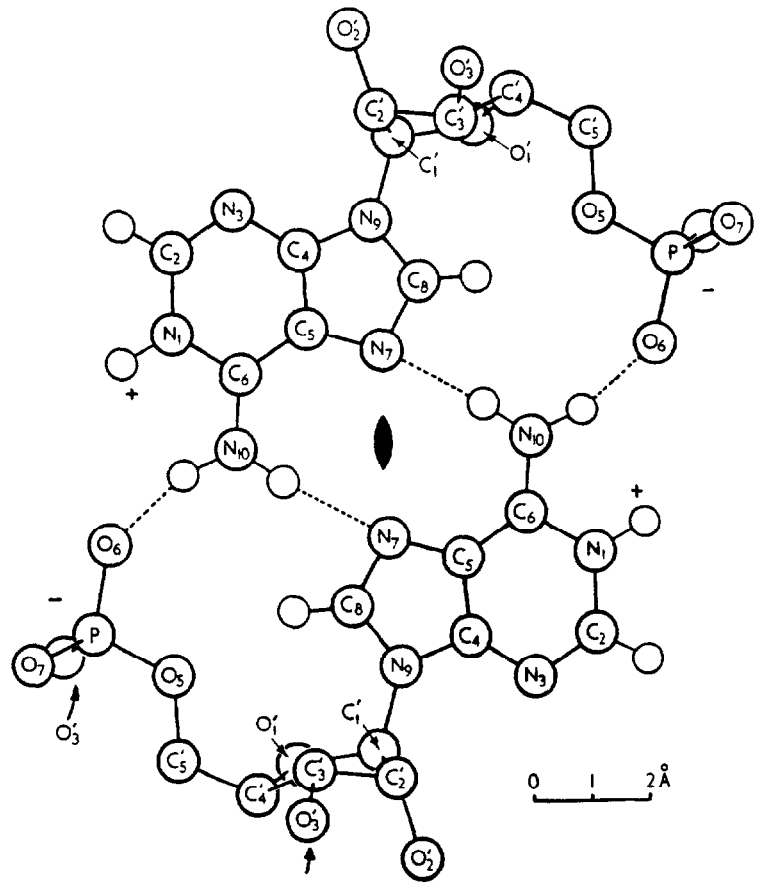

FIG. 5. The two-stranded molecule of polyadenylic acid as viewed down the fiber axis. Each adenine residue forms three hydrogen bonds. An extra O_3' atom is shown to indicate the backbone connections. For clarity the hydrogen atoms on the sugar have been omitted.

represent the adenine residues. Successive residues are related by a translation of 3·8 Å and a rotation of 45°. Both backbone chains are parallel to each other, as shown by arrows. This is in contrast to the two-stranded molecule of deoxyribonucleic acid in which the twofold rotation axes are perpendicular to the fiber axis, and the two backbone chains are anti-parallel. The adenine rings are tilted about 10° from the horizontal, but this is not shown in Fig. 4. Because of the twofold rotation axis, the two bases which are hydrogen bonded to each other are tipped in opposite directions, much like the blades of a propeller.

Figure 5 shows a view of the molecule as seen down the fiber axis. The hydrogen bonding between the adenine residues is shown, as well as the hydrogen bonding

between the adenine amino group and the phosphate oxygen atom from the opposite chain. Two arrows point to successive O_3 atoms of the ribose ring, and that atom is shown twice in order to show how the backbone chain is connected. Nitrogen 1 of the adenine ring is protonated, with a positive charge on the ring. The reasons for this will be discussed below.

In order to see the molecule in terms of the space which it occupies, we have made a diagram in which the atoms are represented as spheres drawn with their van der Waals radii. Figure 6 shows this view of the molecule. The fiber and molecular axis is

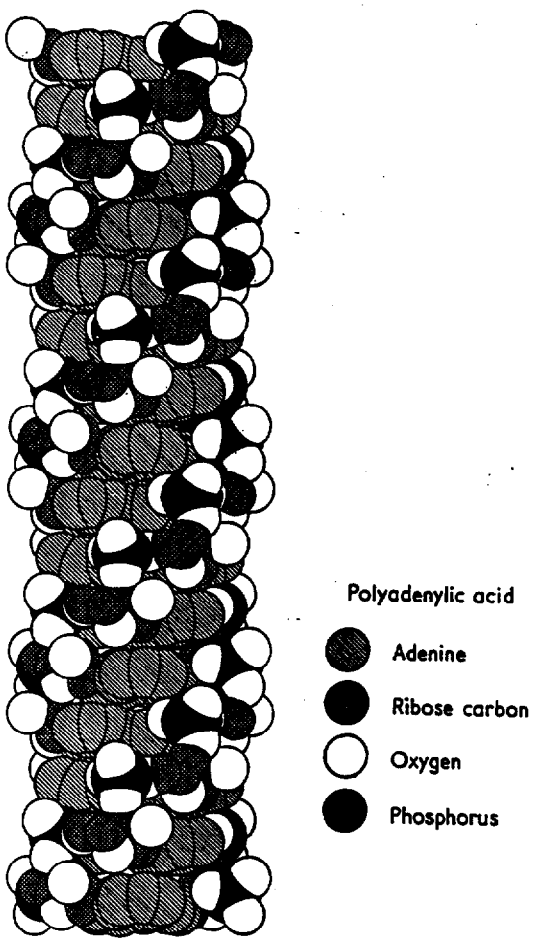

FIG. 6. Van der Waals model of polyadenylic acid. Hydrogen atoms are not represented in this diagram.

vertical, and it can be seen that the adenine bases are slightly tilted. The outstanding feature which this diagram illustrates is the compactness of the molecule. The molecule is a helix, but the helical grooves are not very evident owing to the fact that the phosphate groups are pulled in towards the center of the molecule in order to hydrogen-bond with the adenine amino group.

This compact form is probably responsible for the fact that the helix is intact even under a vacuum. In addition, the rounded outline is probably responsible for randomness in packing, as discussed below.

6. The Density and Space Group of Polyadenylic Acid

To obtain an approximate estimate of the density to be expected we must estimate the number of water molecules that will fit into the unit cell. This can be done approximately by using the partial specific volume of polyadenylic acid in solution, which is known to be in the neighbourhood of 0·54 (R. Haselkorn, personal communication). If we take a tetragonal cell with dimensions $16{\cdot}8 \times 16{\cdot}8 \times 15{\cdot}2$ Å, then calculation shows that there should be room for about eight molecules of water per nucleotide. This gives a density of about 1·47. If the fiber used contained a small amount of sodium ion (which occupies very little volume) this figure might be a little higher, say 1·5.

This is rather lower than the observed value of 1·53 obtained by flotation. This discrepancy can only be explained by assuming that in the fiber there were less crystalline regions with a higher density. As the postulated tetragonal cell is a very open one this is not implausible.

In spite of these uncertainties the density shows clearly that there are two nucleotides for every 3·8 Å in the z direction, since three nucleotides would be an impossibly tight fit (calculated density 1·70) and one nucleotide would give far too low a density (calculated 1·23).

The molecule of polyadenylic acid in the helical form described has a great deal of symmetry. In addition to the twofold rotation axis along the helix axis, there is also an eightfold screw axis, which can be used crystallographically as a fourfold screw axis. Hence the space group may well be $P4_2$ where a c-axis is 15·2 Å and the asymmetric unit consists of two adjoining nucleotides. The helix is covalently linked in a right-handed fashion, but this is not specified by the space group symmetry.

7. Comparison of Calculated and Observed Intensity Data

From the final set of coordinates the continuous Fourier transform was calculated using the computer program already mentioned. This program computes a cylindrically averaged Fourier transform for helical molecules. This is the most appropriate form of comparison to be used for polyadenylic acid since there is little evidence for crystallinity or sampling of the transform with one or two exceptions cited above. The program computes the cylindrically averaged intensity $I(R, l/c)$, where

$$I(R, l/c) = \sum_n \left\{ \left[\sum_j f_j J_n(2\pi r_j R) \cos\left\{ n\left(\frac{\pi}{2} - \phi_j\right) + \frac{2\pi l z_j}{c} \right\} \right]^2 + \left[\sum_j f_j J_n(2\pi r_j R) \sin\left\{ n\left(\frac{\pi}{2} - \phi_j\right) + \frac{2\pi l z_j}{c} \right\} \right]^2 \right\}$$

where j = total number of atoms in the asymmetric unit; r_j, ϕ_j and z_j are the cylindrical polar atom coordinates, n = order of Bessel functions suitable for the helical symmetry. We have used four orders of Bessel function for each layer line; additional contributions could be neglected. In this computation we have not used a temperature factor.

Intensity data were collected from diffraction photographs obtained with the multiple film technique. The intensities of the diffraction maxima were measured with the recording microdensitometer by taking traces which went at right angles to the layer lines as well as along them. This was done because, as a result of the lack of

complete orientation of the fiber molecules, many of the reflexions were arced, with appreciable intensity off the layer lines. Because of this arcing of the reflexions, and because of the limited integrating ability of the recording densitometer, we regard the intensity data obtained as only semi-quantitative. We have, therefore, not considered it worthwhile to correct the recorded intensities for the usual cylindrical and polarization factors. These corrections, if applied, would have the principal effect of reducing the recorded intensities near the meridian.

It was possible to measure the relative intensities of the two reflexions which occur at 1·68 Å and 1·90 Å, but no attempt has been made to relate these accurately to the rest of the diffraction pattern.

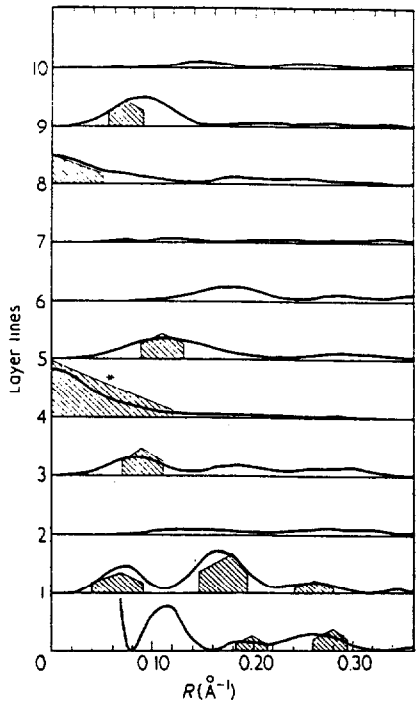

FIG. 7. The observed and calculated intensities for polyadenylic acid. The calculated intensities are indicated by the continuous curves. The observed data are plotted in the shaded areas, the apex indicating the position of maximum intensity. The height of the apex indicates intensity, but the measurements may be in error by as much as 50%. The observed reflexions on layer lines 8 and 9 are not on the same scale as the other observed intensities. *Intensities on this layer line are plotted at one-fifth the actual values.

The observed and calculated intensities on the various layer lines are shown in Fig. 7. It can be seen that there is moderately good but not perfect agreement. Note particularly that the model predicts strong intensity at a spacing of 1·90 Å (second order of 3·8 Å) as well as a strong reflexion at 1·68 Å which should be just off the meridian. This latter reflexion which can be seen in Plate II has an intensity distribution compatible with its being off-meridional.

The observed intensity data are plotted in Fig. 7 in such a way as to indicate its semi-quantitative nature. The most intense part of the diffracted intensity on a layer line was located by projecting the diffraction pattern onto an appropriate Bernal chart. This point was used to determine the position of the apex of the pentagonal

shaded areas which indicate observed intensity. This could be done unambiguously in most cases. However, the medium intense reflexion on the 5th layer line never split into its two off-meridional components. Hence its position on the layer line was determined by assuming that the arc originated on that layer line; this uniquely determines the cylindrical radius R for the reflexion. The same technique was used for the 9th layer line reflexion. The height of the shaded intensity figure was determined by the densitometer tracings, while the width was estimated using both the tracings and visual appraisal. The straight edges on the pentagonal intensity figures do not, of course, represent the shape of the diffracted X-ray beams. In Fig. 7, the observed and calculated intensities for the 4th layer line (3·8 Å) are plotted on a scale 0·2 times that of the rest of the diagram, as indicated by an asterisk. Only the outermost equatorial reflexions are plotted in Fig. 7, since the inner reflexions are discrete and are handled separately.

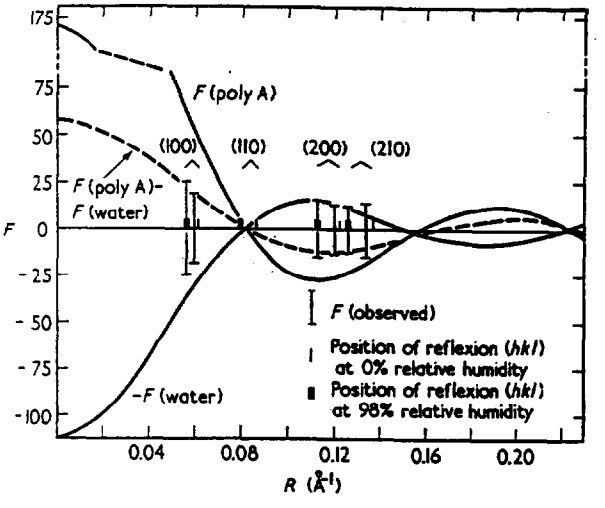

FIG. 8. Observed and calculated structure factors for the equator of polyadenylic acid. The dashed line represents the structure factor for the molecule minus the structure factor for a cylinder of water. The observed data are indicated by vertical lines which cross the horizontal axis. The positions of the reflexions at extreme values of the relative humidity are indicated by short vertical lines.

When considering the agreement between the observed and calculated intensity distributions in Fig. 7 it should be emphasized that no allowance has been made for the effect of bound water molecules on the diffraction pattern. Polyadenylic acid takes up a small amount of water and holds it firmly, and it is quite likely that this water will modify the diffraction pattern slightly.

The water surrounding the molecule may have a substantial effect on the equatorial diffraction pattern. We have accordingly computed the equatorial intensities by taking this water environment into account. This calculation was carried out by assuming that the molecule is cylindrical in outline (cf. Fig. 6), with an outer radius r_0, and is suspended in a sea of water which extends from r_0 to infinity. The equatorial intensities or structure factors are then the difference between the contribution due to the molecule itself and that due to a cylinder of water of radius r_0. Thus, for the equator we have plotted in Fig. 8 the structure factor for the molecule itself, the cylinder of water and the difference between these quantities (dashed line). That is:

$$F = F \text{ (poly A)} - F \text{ (water cylinder)}$$

$$= \sum_j f_j J_o(2\pi r_j R) - \frac{r_o z \rho}{R} J_1(2\pi r_o R)$$

where z = height of the cylinder of water (3·8 Å for 2 nucleotides);
ρ = electron density of water = 0·33 electrons/Å^3;
r_0 = 7·5 Å.

It can be seen that the structure factor passes through zero very near the point at which the 110 reflexion should appear in the square lattice. Hence this accounts for the failure to observe this reflexion. The fact that 210 reflexion is readily observed whereas 200 is frequently very weak may be partially, but not entirely, accounted for by the higher multiplicity of 210. Observed data are plotted in Fig. 8 for the reflexions 100, 200 and 210 at 20% and 98% relative humidity. These structure factor measurements should be regarded as accurate only to approximately ± 30%, due to the diffuseness of the reflexions. However, it can be seen that there is rough agreement with the calculated structure factor curve.

The diffuseness of these equatorial reflexions may arise from several sources. It is quite likely that the molecules tend to crystallize in the tetragonal cell because of the 45° rotation between adjoining nucleotide residues in the chain. However, there are opportunities for various types of randomness. The chains are directional, and so they can be oriented up and down at random. In addition, because the helix is very compact and rounded, rotational disorder can be present. These may have the effect of producing an equatorial packing domain which is small, and leads to broad diffuse equatorial reflexions. This randomness is also responsible for the smeared, non-discrete nature of the off-equatorial reflexions, which come closer to representing the continuous Fourier transform than a sampling at discrete points.

We have spent some time studying the packing between molecules in the tetragonal lattice at various equatorial spacings. There are several possibilities, but we shall not discuss them because the diffraction data are not sufficiently precise.

8. The Model of Morgan & Bear

A paper was recently published by Morgan & Bear (1958) which described two possible structures for the molecule of polyadenylic acid. We believe both of these structures to be incorrect. Since these authors reject their second structure on the grounds that it does not fit the diffraction data, we need not consider it further here. Their structure I is very unusual in that the two polynucleotide chains are wrapped around each other in anti-parallel fashion with each chain containing four residues per turn. The ribose-phosphate chains are located in the center of the molecule with the adenine residues projecting out radially from the center core. The two chains are related by dyad axes, perpendicular to the fiber axis, going through each pair of hydrogen-bonded ribose rings. Although it has some provocative features, this model can be ruled out for several reasons which we shall proceed to outline.

In the first place it is not clear to us that the structure can be built satisfactorily along the lines the authors have described. Whereas most of the bond distances in their model are acceptable, many of the angles are not. In Table 2 we list the bond angles that occur in their structure together with the standard values. It can be seen that distortions are present as large as 20° to 30°, and these are quite unallowable. Furthermore, the authors have neglected to consider some of the unfavorable van der

Waals contacts between various units. Thus, they have the two charged phosphate groups "in contact" with each other. However, the phosphorus atoms are 3·89 Å apart and the oxygens are 2·46 Å apart, both of these values being considerably below the accepted values. We think it is unlikely that these coordinates can be refined to standard values. This is probably an example of the situation discussed above, in that a preliminary and uncritical examination of a potential molecular configuration looks promising but, on closer examination, it turns out that the model is not feasible stereochemically.

TABLE 2

Bond angles in the model of Morgan & Bear

Angle	Value in structure I	Standard value	Distortion
$O_1'C_4'C_5'$	139°	110°	29°
$N_9C_1'O_1'$	132°	110°	22°
$C_2'C_3'O_3'$	124°	110°	14°
$C_1'C_2'O_2'$	120°	110°	9°
$PO_5'C_5'$	102°	about 120°	about 18°
$O_3'PO_7$	77°	110°	33°

Secondly, the structure looks energetically unfavorable. It is held together by one weak hydrogen bond between the sugars, and furthermore, the negatively charged phosphate groups are brought close together so that the molecule would tend to fly apart in solution, whereas it has been shown experimentally by Fresco (1959) that polyadenylic acid retains its helical configuration in solution.

It has been established by Fresco & Doty (1957) that the helical form of polyadenylic acid is stable at pH 5, but not at pH 7. The structure of Morgan & Bear does not explain this. Nor does it explain in a natural way the spacing of 3·8 Å between the bases.

Further support for models having the bases on the inside arises from chemical work concerning the action of concentrated nitrous acid on the adenine residue in ribonucleotide chains. In 4 M-$NaNO_2$ at pH 4·3 adenine residues are usually oxidized to hypoxanthine, since the amino group is replaced by an oxygen. However, polyadenylic acid does not react under these conditions when left at room temperature for four days. (A. Bendich & H. Rosenkranz, personal communication.) This result would not be understandable if the adenine residues project away from the molecular axis into the solution; however, it is naturally explained by the fact that the adenine residues are buried in the center of the molecule and are hence unreactive to nitrous acid.

Finally, it should be mentioned that a cylindrically averaged transform calculation carried out with the Morgan-Bear coordinates does not yield a diffraction pattern in agreement with the observed data. The second strongest reflexion on the calculated pattern is on the 3rd layer line, while the near-meridional part of the 2nd layer line is more intense than the contribution further out. In short, we feel there is very little chance that this model is correct.

9. Discussion

We arrived at our interpretation for the structure of polyadenylic acid before the effect of hydrogen ion concentration was noted. Beers & Steiner (1957) pointed out that an abrupt shift occurred in the ultraviolet absorption spectrum of polyadenylic acid when the pH was lowered to about pH 5. They correlated the shift in the spectrum with the absorption of one proton per nucleotide base. Shortly after that, Fresco & Doty (1957) showed that, at the lowered pH, the molecule had a rigid form composed of variable numbers of polyadenylic acid molecules in contrast to the flexible randomly coiled form which it exhibited at pH 7. Thus we were faced with the question of how the additional proton was absorbed in polyadenylic acid. The answer to this question is quite clear in the case of adenine hydrochloride in crystalline form. Cochran (1951) has made a detailed analysis of the crystal structure of the protonated adenine residue and his results show that the additional hydrogen is located on N_1 of the purine ring. We therefore considered the effects of adding a proton at this position on the structure which we had postulated for polyadenylic acid. The results are shown in Fig. 5 where a positive charge is shown on N_1 in close proximity to the negatively charged phosphate group of the opposite ribose-phosphate backbone. In this position it is clear that the additional charge on the adenine residue stabilizes the molecule electrostatically. This might be described as an "inner salt," since the polymer no longer has an overall charge, even though there are isolated charges on different parts of the helix.

In this form the molecule has considerable stability. It has been shown by Fresco & Klemperer (1959) that the "melting temperature," T_m (the mean of the temperature range over which the material changes from a helical to a random coil form) of a solution of polyadenylic acid at pH 4·25 and ionic strength 0·15 is about 90°C. This is roughly the same as the T_m of calf thymus DNA at pH 7·0 and similar ionic strength, and is considerably higher than the T_m's of polyinosinic acid, polyinosinic *plus* polycytidylic acid and polyadenylic *plus* polyuridylic acid (Doty, Boedtker, Fresco, Haselkorn & Litt, 1959).

Polyadenylic acid, in addition to combining with itself to form this two-stranded molecule, can also combine with polyuridylic acid to form two- and three-stranded structures (Rich & Davies, 1956; Felsenfeld, Davies & Rich, 1957). Similar structures are formed with polyinosinic acid (Rich, 1957*a*) and with polyribothymidylic acid (A. Rich, unpublished results). It should be noted that these structures are all formed at neutral pH where polyadenylic acid exists as a flexible single chain. All of these structures exhibit diffraction patterns that are quite different from that shown in Plate I. However they all have in common a configuration in which the charged ribose-phosphate chains are helically arranged on the outside of the molecule surrounding the hydrogen bonded purine or pyrimidine residues.

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